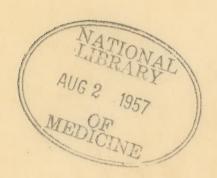
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GENERAL HEADQUARTERS H, S.Army . FAR EAST COMMAND (MILITARY INTELLIGENCE SECTION, GENERAL STAFF).
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Army Medical Gollege Spidemiclogical Research Report

Section 2. Number 377

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> Arry Medical College Spideziology Laboratory (Maj Gen ISHII, Commanding) ENDO, Takeshi Non-official staff

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Major (Medical) HAITO, Ryoichi, Officer in charge. arey Medical College Spidendelogical Research Report

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General

Studies on the antigenic properties of cholera vaccines have progressed remarkably in recent years. Particularly outstanding have been those achievements spurred on by the recent war.

When compared to vaccines of the past considerable improvement is seen in antibody-production especially in the reduction of secondary effects; the antigenic properties of various antigen types are stronger. The supersonic wave-treated cholera antigen is an antigen prepared by subjecting the cholera bacteria to the action of supersonic waves and thereby destroying their cells. Publications on supersonic wave-treated antigens are too numerous to mention. As the results on their agglutinin, bacteriolysin-production, complement fixation substances and immunisation strength proved superior to those of other antigen types, they will be elaborated below in this report.

Chapter I. Inoculation materials and test procedure.

- A. Inoculation materials: The test vaccines consisted of the supersonic wave-treated vaccine (hereafter referred to as U.S.V.) and the control cholera vaccine (hereafter referred to as K.V.) manufactured by this school. Laboratory personnel were divided into two groups and inoculated with each of the vaccines. Comparative studies were performed on antibody productivity within the blood following the inoculation.
- 1. U.S.V. manufacture: The bacterial strains from which the polyvalent cholers vaccine was derived consisted of the Ishii, Ueguchi, Takiguchi, Akatsuka and Imase strains for the original type; the Hikojima and Chosho strains for the intermediate type; and the Dairen 47 and Ogawa 19 strains for the variant types. A 10 mg-per:cc suspension of each strain was prepared with a physiological saline solution after culturing with agar (PH 7.6) at 37°C for 20 hours. Each suspension was subjected to a supersonic wave treatment (560 kc) for a 20-minute period. These were preserved in a refrigerator (4°C) following a PH correction and were employed for the tests five days later.

The suspensions were comprised of 120 cc of the Ishii strain, 40 cc each of the Ueguchi, (Shanghai) Takiguchi, and Akatsuka strains, 80 cc of the Imase strain and 20 cc each of the Hikojima, Chosho, Dairen 47 and Ogawa strains. The bacterial content per cubic centimeter was 10 mg.

The supersonic wave treatment time and the bacterial suspension concentration found to be most satisfactory in the previous experiment were adopted. Virulence against mice (± 12 g) was 0.3 mg. for the Ishii strain, 0.2 mg for the Ueguchi, Akatsuka and Imase strains, over 0.5 mg for the Takiguchi strain, 0.3 mg for the Hikojima strain, 0.2 mg for the Chesho strain and 0.2 mg for the Dairen 47 and Ogawa strains.

- 2. K. V. manufacture: The No. 2 Laboratory was entrusted with the preparation of a polyvalent suspension consisting of a mixture of the nine strains described above. This was stored in a refrigerator after PH correction.
- B. Test procedure: Laboratory personnel were divided into two groups of five persons each (U.S.V. group and K. V. group) and given inoculations of each vaccine type. The inoculation of

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Then every in authbody-production expectally in the reduction of as seen in authbody-production expectally in the reduction of assent as seen in authbody-production properties of various antiquations are types are stronger. The supersents wave-treated chalers antiquation as an author properties to the chales are included by subjecting the choles bestarts to the action of supersents waves and thereby deadneyday their cells. Inditections on supersents wave-treated antique are too macrous to mention in the test tests are their arguments, backericipate to muchocies, supplement firmtion reductions antique the insumination are too mention proved superior to these of other antiques types, they will be also antique that request.

Chapter I. Isosolation nathrates and test promodure.

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The suspensions were described of 180 on of the loais strain, 40 or each of the Seguchi, (Shanghai) Tabigashi, and Akatanka strains of the test lead strain and 20 or each of the Sikajias, Chosho, Dairon 47 and Ogash strains. The backerial combont per cohold confidence was 10 mg.

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2. K. V. menufacturer fine No. 2 Laboratory was entracted with the properties of a prigrains described above. This was atored in a safety see after 18 corrector after 18 correctors.

is feat procedure: Laboratory personnel were divided into two groups of five persons mask (E.S.V. group and M. V. group) of and persons of each vecular type. The insculation of

the U.S.V. group was completed successfully but unfortunately one person in the K.V. group became ill and another retired from the service during the course of the inoculation.

The presence of healthy antibodies was determined by taking blood before the inoculation. Preliminary tests on healthy sers consisted of the determination of agglutination, complement fixation test, test-tube bacteriolysis and immunization tests.

The first ineculation series consisted of 4.0-ag doses injected subcutaneously on the upper arm on 10 Apr 40; the second series (6.0-ag doses) being performed on 18 April. Serum was separated and readied for tests on the minth day (25 April) following the second ineculation series.

Antibody productivity following the injections was examined by means of agglutination reactions, complement fixation reactions, test-tube bacteriolysis and immunization tests.

1. Agglutination reaction: The test followed orthodox practices. Serum dilution started with a five-time dilution for the first test tube and ended with a 1,280-time dilution.

The antigens were 1.0-cc and 0.3-cc bacterial suspensions of the Ishii, Hikojima and Ogawa strains each of which was cultured in agar for 20 hours at 37°C and diluted with a sterile physiological saline solution. One cc of each antigen was used for the above serum. After being kept in an incubator for two hours at 37°C and left standing overnight at room temperature the results of each were observed and evaluated.

2. Complement fixation reaction: The test was based on the Kobayashi method. As a preliminary test, the hemolytic titer and the entigen-complement titer were measured. Hemolysins were of the goat series possessing a hemolytic titer of 6,400 times. Four units were used for this test.

The complement was derived by separating the sera taken from 10 marmots and used after standing for three hours at room temperature. The volume of the complement to be used was measured each time. Dilutions ranged from 10 to 16 times. A test for anti-complementary effects was conducted before use.

The use of the U.S.V. antigen followed the procedure outlined in previous reports. The nine bacterial strains were cultured in agar (PH 7.6) for 20 hours at 37°C and suspended in a physiological saline solution at a ratio of 10 mg per cc. These were treated with 560-kc supersonic waves for 20 zimutes. This was followed by the addition of 0.5 per cent carbolic acid and by refrigeration at 4°C. The antigens displayed a "self-retarding action" at a dilution of eight times when the fixation titer against immune sera was measured. It was proved that fixation in immune sera and human sera was adequate.

Test ears were rendered inactive (56°C, 30 minutes) prior to the test.

In the main test, 0.4 ec of the test sarum and 0.6 ec of a physiological saline solution were placed in the first test tube and 0.5 ec of a physiological saline solution was used in diluting the contents of the second and subsequent test tubes.

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 One half cc each of the antigen was poured into the second and subsequent test tubes and 0.5 cc each of the complement was added, starting with the first test tube. Those were thoroughly shaken and placed is an incubator for one hour at 37°C. This was followed by the addition of 1.0 cc of a 5.0 per cent sensitized blood cell solution and a two-bour incubation at 37°C. The results were evaluated the following morning after the contents were stored in a refriverator.

- 3. Past-tube bacteriolvais: The test sera were inactivated before use: the complement was derived from markets. The bacterial solution employed was premared by a 37°C, 20-har culture and indicated a colony count of 1,000 per de when diluted to 10° times with bouillon in a Petri dish. This test was deped on the baisser—Vechebers method. The dilution for the last test tube. The test about the a 2,560-time dilution for the last test tube. Due to a shorter of initial blood sera all bacterial types could not be tested (test overed only the original type). Tests covering every type were possible with the final blood sera.
- 4. Immiration test: The original, intermediate and variant types were cultured in a per for 20 hours. The developed bacteria were suspended in a physical ical satine solution at a ratio of 0.7 mg/0.4 cc for the original type and the intermediate type and 0.5 mg/0.2 cc for the variant type. Esploying a taberculin by odermic syrings 0.1 cc each of the test serve and 0.4 cc each of the bacterial solution were drawn and intraperitoneally injected into a German mouse (12 g). Fice were observed for a tiree-day period.
- C. Inocalation reaction: Post-inocalation symplems of a constitution I nature such as calls, favorishmes, vertice, heaviness of head, general fations, arthraldia of the extractiles, oppressive mains and polyic price and those of a local nature such as inflamention, swalling, "soontmesus sales" and oppressive mains were recorded. It is a vertices accurring only once during the one-week observation period following the injections were recorded as being positive.

Chapter II. Tost results.

- A. Togethe of experimentation on various artibodies in healthy server "seliho as helicotion reactions, bacterialysis, complement firstion reactions and improvention tooks were performed on blood draw from seven errors. The retained assent of acti-bodies is reported below.
- 1. The let be beauthy as lutining The explicited processor results on the healthy as lutining from sive 0.1.7. cases showed for the crisinal type, three cases positive at 5-10 times, two cases positive at 20 times, and one case positive at 40 times. Five cases of the variant type were positive at 5, 10 and 20 times, four cases at 40 times and two cases can at 40 and 160 times. The original and intermediate types were positive at 40 times and the variant type at 160 times.

Healthy agglutinin was retained at 40 times and below but rerely at 160 times. (See Figure 2.)

arrhytimin was absent in the K.V. original type. One case of the intermediate type was positive at 40 times. One case

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of the intermediate type was positive at 20 times. One case each of the variant type was positive at 10-20 times (\pm). Extremely small amounts of healthy asclutinin were present. (See Figure 3.)

2. Femilts on test-tube bacterialysis: Bacterialytic reactions on the W.S.V. cases were limited to the initial sera of the original type. The results disclosed four cases indicating positive bacterialysin at 10 times and two cases at 20 times before W.S.V. inoculation. The remaining two cases were negative. Consequently, bacterialysin retention in healthy blood was indicated at 20 times.

The original and intermediate types of initial K.V. sera were negative at 5 times. The variant type was positive at 5 times. Bacterial growth was unlimited in the others.

- 3. Complement fixation reaction results: Complete hemolysis compared to the control was produced at 5 times. Three cases were positive at 10 times.
- 4. Immunication test: The initial U.S.V. sere were positive for two cases of the original type and one case of the intermediate type. The initial K.V. sera were positive for one case of the intermediate type and two cases of the variant type. In short, the defensive strength of mice against healthy sera was weak in the case of the intermediate type and the variant type.
- B. Constitutional and local symptoms following inoculation: Secondary effects following the inoculations appeared to have been greatly influenced by the type and the individual characteristics of the anticen. The constitutional symptoms following the first injection series of U.S.V. were four cases of feverishness (approximately 0.5-1.700) and general fatigue (all cases). Beaviness of the head (nervous symptom) was indicated by three cases.

One case each in the K.V. group showed general fatigue and loss of appetite. Reaviness of head and headaches (nervous symptoms) were indicated by one case each. The incidence of general fatigue and feverishness was higher than that contained in the initial report (July 1974). Local symptoms such as inflammation, swelling, "spontaneous pains" and oppressive pains were displayed by both vaccines.

Secondary effects such as inflammation, swelling, "spontaneous pains" and oppressive pains were stronger compared to those shown by results in the initial report. A sharp reduction in secondary effects compared to the first series was observed following the completion of the second injection series. One case in the U.S.V. group developed inflammation, thirst, diarrhea and headaches when inoculated following an attack by a cold. The others, however, indicated only general fatigue and heaviness of head (two cases). Post-injection local symptoms such as inflammation, swelling, "spontaneous pains" and oppressive pains were positive in every case.

Consequently, both groups displayed, as a secondary effect, severe local reactions as well as constitutional symptoms such as general fatigue, inflamation, feverishmess, headerhes and heaviness of head, the intensity of which decreased considerably in the second injection series. (See Fable 1.)

The foregoing results indicate the necessity for performing general studies on the detoxication of the U.S.V. vaccine.

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- C. Serological observations following inoculation: The second inoculation series followed the first series after a one-week interval. Quantitative tests were conducted on the antibody productivity of blood taken a week following the completion of the second series.
- 1. Application productivity test results: According to OKT SU against for the U.S.V. antisen indicated maximum positivity at 900 times on the seventh day and at 1,200 times on the tenth day. The F.V., however, was positive at 320 times for the original, intersectate and variant types, differing markedly from the U.S.V.

The following observations on agglutinins are classified according to the antigen type:

a. U.S.V.

Original type: Five cases each positive at 5-320 times; four cases at 540 times and one case at 1,200 times. Negative at 2,560 times.

Intermediate types Five cases each positive at 5-160 times; four cases at 1,260 times. Negative at 2,560 times.

Variant type: Five cases positive at 5-440 times; four cases at 1,200 times and two cases at 2,500 times. (Dee Figure 2.)

b. K.V.

Original type: Two cases positive at 5-320 times.

Intermodiate type and variant type: Two cases positive at 5-80 times and one case at 160-330 times. Regative at 640 times. (See Figure 3.)

The results of acristinia productivity tests performed on the above are mumuarized below:

- (1) As alutinin for the U.S.V. was greatly superior to that for the U.V. As lutinin was positive at 160 times in case of the univalent antigen mentioned in earlier reports (April 1937, section dealing with inoculations). As slutinin-production in the blood as ainst polyvelent antigen was positive at 1,200-2,560 times which is the true titer for supersonic wave-tranted antigens.
- (2) The sera indicated maximum positivity at 160 times before inoculation and at 2,560 times after inoculation, an increase of 16 times.
- (3) The initial Y.V. sers indicated positivity at 20 times. Fositivity was displayed at 320 times after inoculation, an increase of 16 times.
- U.S.V. value was 2,560 times (eight times higher).
- c. Test results on an intimation titers showed, when combining the titers for three antibody types in the initial U.S.V.

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seru, 12 cases each at 5-10 times, nine cases at 20 times, six cases at 40 times and two cases such at 80 and 160 times.

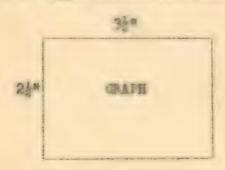
In the case of the final sers, there were 15 cases each at 5-160 times, 14 cases at 320 times, 13 cases at 640 times, seven cases at 1,200 times and two cases at 2,500 times.

The initial K.V. sars showed two cases at 5-10 times and one case at 20 times. The final sars indicated six cases such at 5-20 times and four cases each at 160 and 320 times.

d. Figure 4 is a greatic interpretation of an intination titors. The mark for the initial U.S.V. sorn is between 4 to 10 times. The curve gradually descends from the fo-time point.

The final sers continue on the same level from 5 to 160 times and descend redually, starting from 320 times and ending at 2,860 times. (Coe Figure 4.)

Figure 4. Applytination results



Koy

- (1) _____ Initial U.S.V. sera
- (2) Final W.S.V. sore
- (3) o--- Initial K.V. sera
- (4) o--- Final K.V. sera

The peak for the initial F.V. sers continues from 5 to 10 times before the curve descends. The final sere hold a peak at 5-20 times, the curve crop in abarply at 160-320 times.

The continuous straight line indicated by U.S.V. is a sire of its superior properties.

exponents of evalutination times is 12 points at 10 times, 18 points at 20-40 times, eight points at 80 times and 12 points at 160 times or a total of 68 points. The final serus score is 15 points at 10 times, 30 points at 20 times, 25 points at 40 times, 60 points at 80 times, 75 points at 161 times, 84 points at 320 times, 84 points at 640 times, 56 points at 1,200 times and 18 points at 2,560 times or a total score of 467 points.

The initial F.V. sorum score is two points at 10 times and three points at 20 times or a total of five points. The final serum score is six points at 10 times, 12 points at 20 times,

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18 points at 40 times, 24 points at 80 times, 20 points at 160 times and 24 points at 320 times or a total of 104 points.

When comparing the scores of U.S.V. and N.V., the former is roughly four times higher than the latter. (See Figure 5.)

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Figure 2. Test results in U.S.V. inoculation

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Figure 5. Agglutination reaction exponential table

Serum dilution	a (x)	(—)	5	10	20	40	80	160	320	640	1280	2560	Total
Number positive U.S.V.	Initial	0	12	12	9	6	2 15	2 15	0 14	0	0	0	metric no eta la metro devolunto, il cun
Number positive R.V.	Initial Final	0	2	2	1 6	0	0	0	0	0	0	0	D and a milliouring attrinue seed of a first princip
U.S.V.	Initial	0	0	12	18 30	18	8	12 75	0 84	0 84	0 56	0	68
K.V.	Initial Final	0	0	2 6	3	18	9	0 20	24	0	0	0	5 104

- 2. Results of test-tube bacteriolysis: A high bacteriolysis titer was the desired result in bacteriolysis but the growth of polyvalent antigens proved to be inadequate for all bacterial types. The results are enumerated below.
- a. Bacteriolysin production in the blood following inoculations of U.S.V. antigen was noted. Bacterial growth was indicated by one case of the original type at 320 times and by three cases at 640 times. Bacteriolysin was not retained by five cases at 1,280 times and above. The intermediate type showed one positive case at 160 times, one case at 320 times and three cases at 640 times; all cases were positive at 1,280 times and above.

Bacteriolysin production was detected in the blood following the K.V. inoculations. Racterial growth for the original type was present in one case each at 40 times and 320 times. The intermediate type was positive at 20 times (one case) and 160 times (one case). The variant type was positive at 2 times (TN: Sie). Consequently, this vaccine possesses bacteriolysin at 40 times.

A comparison of the U.S.V. and the K.V. is presented below.

- (1) The initial U.S.V. serum (original type) is positive at 20 times and the final serum is positive at 160 times. The variant type is positive at 1,280 times. In short, the bacteriolysin production of the final serum is 8-64 times that of the initial serum.
- (2) The production indicated by the original, intermediate and variant types of K.V. are four times those of U.S.V.
- (3) The bacteriolytic titer of the intermediate types for both U.S.V. and K.V. is one step lower than that of the original and variant types.

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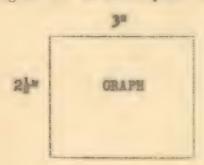
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(4) The values indicated by the final U.S.V. serum are four times higher than those of the initial serum.

b. A graphic representation of the bacteriolytic titers reveals sharp fluctuations in the initial U.S.V. and the initial and final K.V. curves up to the 40-time point. In other words, bacterial growth occurs and bacteriolysin production ceases beyond this point. On the other hand, the bacteriolytic titer curve for the final U.S.V. serum describes a steep slope between the 160- and 640-time points. This shows that the bacteriolytic titer of the U.S.V. is overwhelmingly superior. (See Figure 6.)

Figure 6. Bacteriolysis results



Key

- (1) Initial W.S.V. serum
- (2) -- Final U.S.V. serum
- (3) 0--- O Initial K.V. sarum
- (A) o--- Final K.V. serum

c. The secres for the logarithmic exponents of bacteriolytic titers are 172 points for the initial U.S.V. serum and 317 points for the final U.S.V.; and 225 points for the initial K.V. serum and 235 points for the final K.V. serum.

Figure 7. Bacteriolysis exponential table

Serum dilution	a (m)	5	10	20	40	80	160	320	640	1280	2560	fotal
Number positive	Initial	0	0	2	4	4	4	4	4	4	4	
U.S.V.	Final	0	0	0	0	0	1	3	9	12	15	
Number positive	Initial	4	5	5	5	5	5	5	5	5	5	
K.V.	Final	0	0	4	12	16	20	24	28	32	36	
U.S.V.	Initial	0	0	4	12	16	20	24	28	32	36	172
Secre	Final	0	0	0	0	9	5	18	63	66	136	317
K.V.	Initial	0	5	20	15	20	25	20	35	40	45	225
Score	Score Final		0	2	12	16	25	36	42	48	54	235

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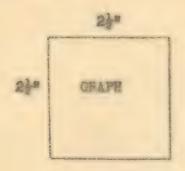
3. Results on productivity of complement fixation products: The superiority of the supersonic wave-treated antigen in producing complement fixing antibodies following its inoculation has already been established in reports published by this and other laboratories. However, studies concerned with fination tests on polyvalent, mixed antigens have not been reported. The comparative studies made on U.S.V. and K.V. are described below.

a. Three cases of the initial U.S.V. serum (five times control) were positive at ten times. The final serum (five times control) was positive for five cases each at 10 and 20 times, three cases at 40 times, two cases at 80 times and one case at 160 times.

Hemolysis was present in every case with the initial K.V. serum. The final serum (five times control) was positive for two cases each at 10 and 20 times and for one case each at 40 and 80 times. The difference between U.S.V. and K.V. was extremely slight, their raties being 3.2:3.0. Post-inoculation antibody production occurred at 170 times compared to 10 times before inoculation.

b. Both U.S.V. and K.V. describe a sharp peak between 10 and 20 times when represented graphically. The U.S.V. curve drops gradually from this point down to the 160-time point. The K.V. curve drops abruptly, starting from the 20-time point and ending at the 80-time point. (See Figure 5.)

Figure 8. Complement fixation reaction results



Kay

- (1) Final U.S.V. serum
- (2) o--- Final K.V. marun

e. The scores obtained from the locarithmic exponents of complement firstion reactions are, in the case of the final U.S.V. serve, five points at 10 times, 10 points at 20 times, nine points at 40 times, eight points at 50 times and five points at 160 times, or a total of 37 points. The scores for E.V. are two points at 10 times, four paints at 20 times, three points at 40 times and four points at 80 times, or a total of 13 points. The U.S.V. in this respect is superior. (See Figure 7.)

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Figure 9. Exponential table of complement firmtion titers

Serum dilution	(x)	5	10	20	40	80	160	320	640	1280	2560	Total
Number positive	Initial	9	3	O	0	0	9	0	0	0	0	
5.8.V.	Final.	9	5	5	5	3	2	1	0	9	0	
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K.V.	Final	ล	2	2	1	1	9	0	2	9	0	
U.S.V.	Initial	9	3	?	0	9	0	9	0	0	0	3
acore	Pinal	9	5	10	9	8	5	0	0	0	9	37
R. A.	Initial	9	9	0	1	0	9	9	0	0	0	0
seare	Final	9	2	4	3	4	9	9	0	9	0	13

A. Immigation test results.

a. No deaths occurred during the three-day observation period following the injection of mice with the final U.S.V. serum. In the test, one-unit (0.7 mg) and two-unit (1.4 mg) doses of the original type failed to produce death. The same results were obtained with equal doses of the intermediate and variant types.

Two rice (variant type) and (Dis Figure missing)
mice (original and intermediate type) died on the first day
following the injection of final E.V. sera.

b. One dooth each was produced on the first and second day with initial U.S.V. sera of the original type; one death occurred on the third day with the intermediate type. One death per day resulted with each W.V. sera of the original, intermediate and variant type. Three deaths occurred out of the eight cases treated with the foregoing initial U.S.V. sera. Three out of six cases died from doses of initial W.V. sera. The final U.S.V. serum caused one death out of a total of 15 cases. Three deaths out of six cases resulted with the final W.V. serum. (See Figure 10.)

Figure 10. Immunipation test results

Observation	ported	let	2nd day	3rd day	Total	Number of times
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positive U.J.V.	7 inal	The second secon	0	5	0	15
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positive K.V.	Finel	3	ð	0	3	6
Total	Initial	4	1	1	6	в достоя в достоя дал, в стоя в вой в подовой в под В ДД
	Final	3	0	0	3	21

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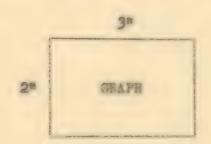
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e. Through a graphic illustration of the test results it will be observed that the initial U.S.V. serum indicates a value of 2.5 per cent (IN: Sic) over the entire period and that E.V. indicates 50 per cent on the first day. The value for the final U.S.V. serum is negative. The final E.V. serum indicates 50-per cent fatality (three cases).

As a result the superior features of the U.S.V. and the K.V. were determined on the third day of observation. (See Figure 11.)

Figure 11. Immunisation test results



Key

- (1) lst day
- (2) 2nd day
- (3) 3rd day
- (4) 1st day
- (5) 2nd day
- (6) 3rd day
- (7) Three-day period
- (8) Three-day period
- (9) Initial sera
- (10) Final sera
- (11) Initial sers
- (12) Final sera

Summary and Conclusions

Comparative experiments were performed on the active antibody productivity of polyvalent U.S.V. and H.V. cholera antigens. The fact that antibodies produced by the univalent U.S.V. antigen are superior to H.V. antibodies has already been reported. This has been established by the results of experiments on antibody productivity before and after inoculation. In the experiment the inter-relationship between the antibodies (in the blood) of the

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A. The secondary effects following an inoculation of U.S.V. vaccine are feverishness and general fatigue coupled with local reactions such as inflammation, swelling, "spontaneous pains" and oppressive pains. Approximately similar results are produced after the second injection series (one case displayed loss of appetite, thirst and hasdaches as well).

The symptoms displayed by K.V. cases are general fatigue, loss of appetite, headaches and heaviness of the head. Local reactions include inflammation, swelling, "spontaneous pains" and appressive pains. Reactions in the second injection series are practically confined to those of the localized type.

In view of the above results, the lessening of the secondary effects of U.S.V. presents an urgent problem.

- B. Healthy sera indicate an agglutination titer of 40 times for the original and intermediate types and 160 times for the variant type; bacteriolysin at 20 times; complement fixation reaction at 10 times; and positivity to immunization on the second day. The maximum agglutinin-antibody production for each final U.S.V. serum type occurs at 1,280 times (one case) for the original type, 1,280 times (two cases) for the intermediate type and 2,560 times (two cases) for the variant type. Maximum production in the case of the K.V. serum occurs at 320 times (two cases) for the original type and at 320 times (one case each) for the remaining types.
- C. Bacteriolysin retention is observed in every type of initial U.S.V. sera at 20 times (two cases). Bacteriolysin is present in the final sera at 160 times (one case) for the original and variant types and at 80 times (one case) for the intermediate type. The initial K.V. serum is positive at five times (one case) and the final serum at 10 times (six cases).
- D. The initial U.S.V. serum is positive at 10 times (three cases) when tested for complement fixation; the final serum is positive up to 160 times (one case). The final K.V. serum is positive at 80 times.
- E. Immunization tests reveal three deaths with initial U.S.V. sera but none with the final sera; and three deaths each with the initial and final K.V. sera. This proves that the immunization strength of U.S.V. is high.

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